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ORIGINAL ARTICLE

EFFECT OF DIFFERENT COMBINATIONS OF BENZYL ADENINE AND NAPHTHALENE ACETIC ACID ON MICROPROPAGATION OF *CARICA PAPAYA* L. HYBRID PLANT *IN VITRO*

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Abstract: The experiment was conducted in Fadak Company in tissue culture labs, Basrah, Iraq, to study the effect different concentrations of benzyl adenine and naphthalene acetic acid, *in vitro* (papaya hybrid). The treatment combination of MS medium + 1.0 mg L^{-1} BA and 0.5 mg L^{-1} NAA has been recorded the highest number of shoots, reached to 4.7 shoot $explant^{-1}$. The treatment combination of MS + 0.5 mg L^{-1} BA + 0.5 mg L^{-1} NAA gave the lowest mean number of shoots with 1.6 shoot $explant^{-1}$. The treatment combination of MS + 0.5 mg L^{-1} BA + 0.5 mg L^{-1} NAA has achieved the best number of leaves per shoot, reached to 3.9 leaves $shoot^{-1}$. The treatment combination of MS + 1.0 mg L^{-1} BA + 0.5 mg L^{-1} NAA has been recorded a high length of shoot, reached to 1.06 cm. The highest fresh weight of the shoots was 0.172 g recorded by the treatment combination of MS + 1.5 mg L^{-1} BA + 0.5 mg L^{-1} NAA. While, the treatment combination of MS + 1.5 mg L^{-1} BA + 1.0 mg L^{-1} NAA achieved the highest dry weight mean of shoot reaching to 0.052 g.

***Key words*:** Cytokinin, Explant, Initiation stage, Shoot proliferation.

Cite this article

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1. Introduction

Papaya (*Carica papaya* L.) plant is an economically important fruit tree in tropical and sub-tropical regions. It is a fruit native to southern Mexico and South America [Dixon (2007)]. Papaya has been a major commercial crop for the past few years due to its high nutritional value. One of the main advantages of papaya is that the fruits yield year-round fruiting unlike other perennial crops [Crane (2013)]. Papayas are male, female and hermaphrodite plants [Solórzano-cascante *et al*. (2018)]. The sexual reproduction of the papaya plant by seed is the preferred method of propagation by farmers because it is comparatively inexpensive [Bhattacharya and S.S. Khuspe (2001)]. However, this traditional method has many negative aspects, including genetic heterogeneity, the sexual difference between individuals and the production of non-original species [Costa *et al*. (2019)]. Also, this traditional method of propagation can spread disease infections to new generations on papaya farms [Al-Shara *et al*. (2018)]. Moreover, it is not possible to determine the sex of the plant during the stages of growth until the flowering stage. Therefore, the planted seeds will produce three types of plants (female, male and hermaphrodite) whose sex will be determined at the flowering stage, causing a significant increase in production costs [Bindu (2015)]. The propagation problems required replacing the method of propagation by seeds with a modern vegetative propagation technique, which is the propagation of plant tissues of the desired female and hermaphrodite plants of papaya trees. Tissue propagation is the only economical method by which papaya plants, similar to the selected mother plant, which has high-quality and quantity yield [Ragavendran and Natarajan (2017), Al-Shareefi *et al*. (2020)]. Several studies have been conducted on the

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micropropagation of papaya by in vitro technique, to obtain plants similar to the mother plant with high yielding traits in qualitative and quantitative terms [Kanth *et al*. (2017), Podikunju (2017), Rohini (2017)].

Phytohormones are the most important endogenous substances for moderating physiological and molecular responses, a critical requirement for plant survival, Phytohormones act at their site of synthesis or elsewhere in plants following their transport [AL-Taey *et al*. (2018), Lateef *et al*. (2021)], Cytokinins encourage cell division and regulate budding. It affects the polarization of mineral elements and nutrients [AL-Taey and Majid (2018), AL-Taey *et al*. (2021)], Benzyl adenine A is the first synthetic cytokine that stimulates plant growth and development, and fruit setting and promotes fruit richness by initiating cell divisions [Hamza and AL-Taey (2020)].

Auxins are phytohormones with essential roles in plant growth and development, including morphogenesis, germination, Vascular development, roots development, apical dominance and cell enlargement, Naphthalene Acetic Acid (NAA) belongs to synthetic sorts of auxins, which play a key role in cell elongation, division, vascular tissue, differentiation, root initiation, apical dominance, leaf senescence, leaf and fruit abscission, fruit setting and flowering [Al- Duraid *et al*. (2019), Safana *et al*. (2022)].

Among the factors affecting the micropropagation of plants are growth regulators, which take place through the balance between growth regulators added to the nutrient medium and plant hormones. The balance between auxins and cytokinins is often essential for plant tissues grown by in vitro culture technique. The growth and development of adventitious shoots and roots are stimulated. This study was conducted with the aim of micropropagation of hybrid papaya plants with desirable traits by studying the different combinations of BA and NAA on some growth parameters.

2. Materials and Methods

The experiment was conducted in the Plant Tissue Culture Labs. of Fadak Private Company, Abu Al-Khasib District, Basrah Governorate, Iraq. The explants (axillary buds) were obtained from hybrid papaya seedlings at three months (Plate 1, A, B). The shoots were excised with a sharp blade. Then the excised buds were sterilized for ten minutes with mercury chloride ($HgCl_2$) at a concentration of 0.01% and several drops of Tween 20 has been added to the solution too. After that, it was washed with sterile water five times to remove the effect of the mercury solution from the surfaces of the explants.

The nutrient medium prepared for the cultures in the initiation stage consisted of 4.43 g L^{-1} MS salts and vitamins [Murashige and Skoog (1962)] supplied with 0.5 mg L^{-1} IBA + 0.1 mg L^{-1} NAA + 30 g L^{-1} sucrose + 8 g L^{-1} agar [Patel *et al*. (2013)]. The MS medium's pH level containing all of the above was adjusted to 5.8 by 0.1 N HCl or NaOH. An autoclave sterilized the medium at a pressure of 15 lb $inch^{-2}$ and for 18 minutes at 121 degrees Celsius. After that, the sterilized axillary buds were cultured into culture jars containing sterilized MS media. Then, the culture jars were transferred to the growth room at 25 ± 2°C, with light duration of 16 hours day^{-1}. Illumination was provided by a white fluorescent LED.

2.1 Effect of different benzyl adenine and naphthalene acetic acid combinations on the shoot proliferation

The nutrient medium was prepared for the proliferation stage consisting of the 4.43 g L^{-1} MS salts and vitamins supplemented with 30 g L^{-1} sucrose + 40 mg L^{-1} adenine sulfate and different combinations of BA at 0.5, 1.0 and 1.5 mg L^{-1} and NAA at (0.5, 1.0 and 1.5 mg L^{-1} (Table 1). The MS medium pH was adjusted to 5.8 by using the HCl or NaOH solutions at 0.1 N concentration. An autoclave was used to sterilize the MS medium under pressure (15 lb $inch^{-2}$) and 121°C for 18 minutes. After sterilization, the explants produced

Table 1: The different combinations between BA and NAA that added to MS media.

Combination	BA concentration (mg L^{-1})	NAA concentration (mg L^{-1})
T_1	0.5	0.5
T_2	0.5	1.0
T_3	0.5	1.5
T_4	1.0	0.5
T_5	1.0	1.0
T_6	1.0	1.5
T_7	1.5	0.5
T_8	1.5	1.0
T_9	1.5	1.5

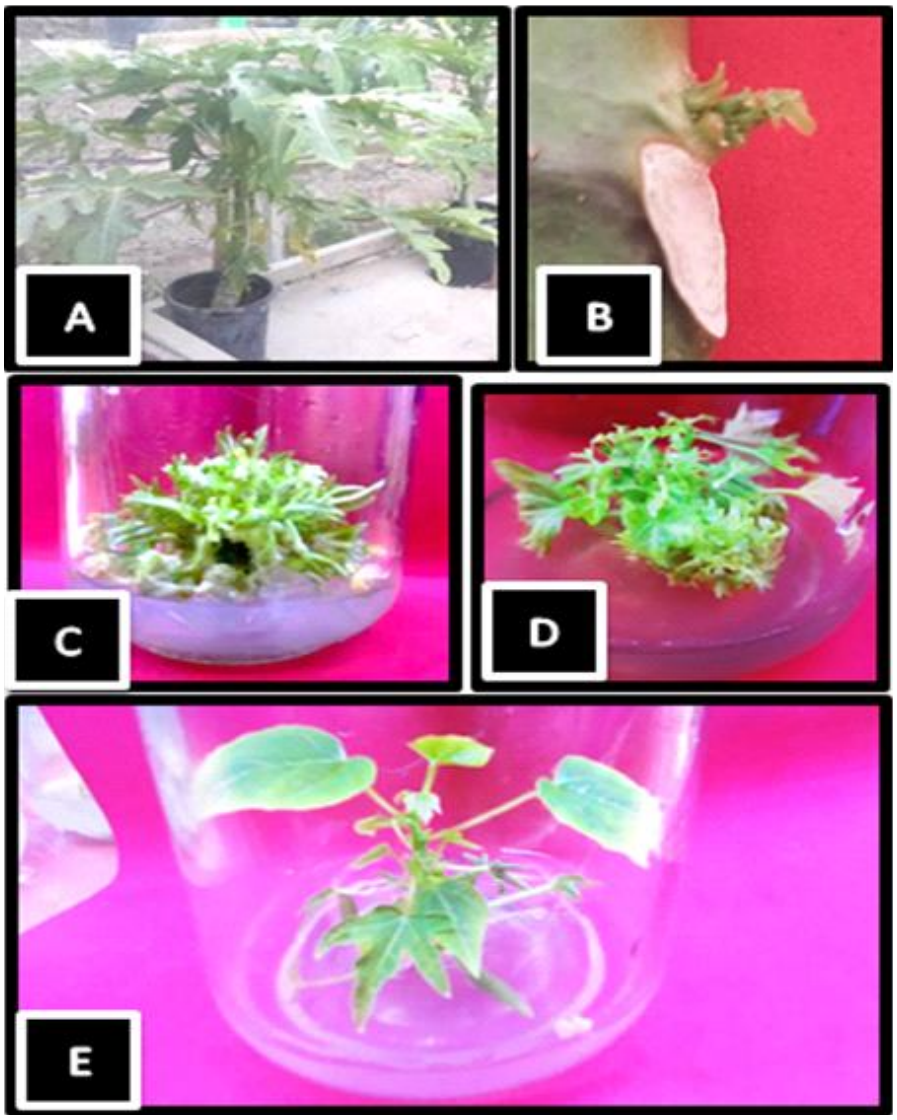

Plate 1: Micro propagation of hybrid papaya plant. A- Hybrid mother papaya plant, B- Axillary bud explant, C- Initiation stage, D- Shoot proliferation stage, E- Proliferated shoot

from the initiation stage were cultured on a medium of MS for shoot proliferation (Plate 1, C, D, E). The culture jars were transferred to the growth chamber at 25 ± 2°C, with a light duration of 16 hours day^{-1}.

2.2 Experiment design and statistical analysis

A completely randomized design has been conducted for this experiment. The data has been statistically analyzed. The means of the treatments were compared using the least significant difference test at the 5% probability level [Snedecor and Cochran (1980)]. Each treatment has been repeated ten times. Observations have been recorded for each experiment every four weeks after it started.

2.3 Experimental Measurements

1. The response of explants to the shoot proliferation (%)

2. The number of formed shoots per explant (shoots explant^{-1})

3. Shoot length (cm)

4. Number of leaves per shoot (leaves shoot^{-1})

5. Fresh weight of the shoot (g)

6. Dry weight of the shoot (g)

3. Results and Discussion

Fig. 1 indicates that treatments T_4, T_5, T_7, T_8 and T_9 achieved the highest response of branch formation 100%. While, the treatment T_6 recorded the lowest value in the emergence of branches, amounting to 60%.

Fig. 2 indicates a significant difference in T_4 treatment compared to other treatments based on the

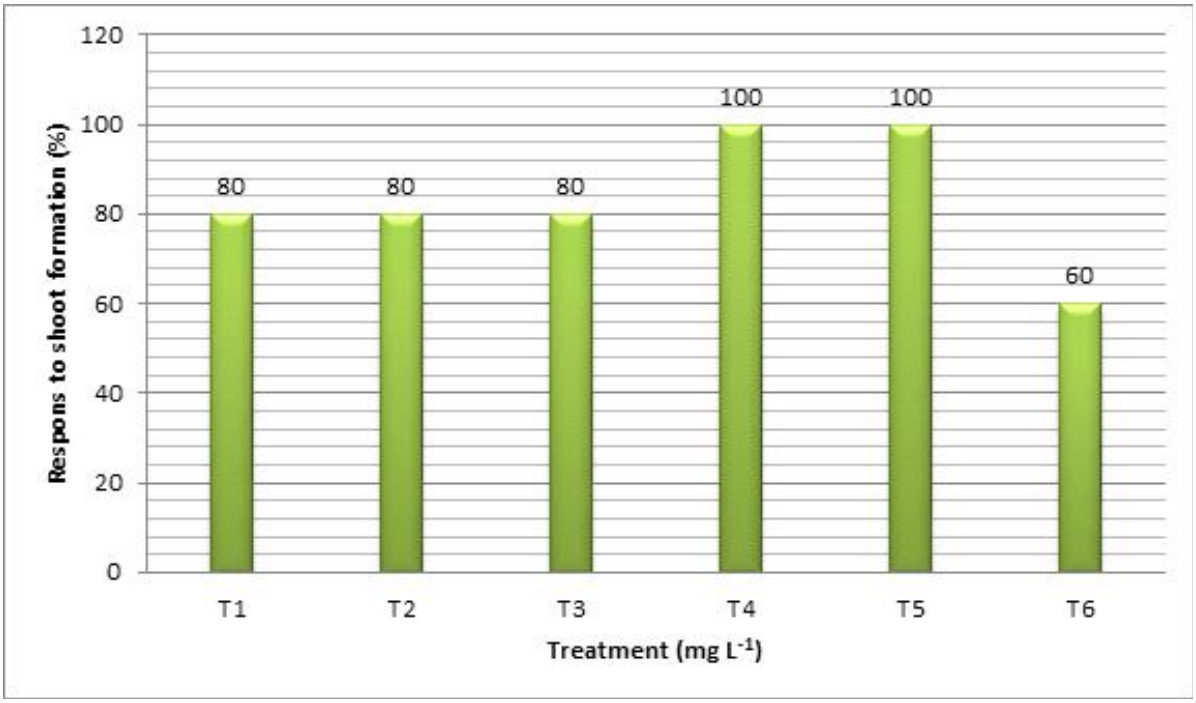

Fig. 1: Effect of different combinations of BA+NAA on the response of explant to shoot formation (R-LSD p ≥ 0.01 = 0.352)

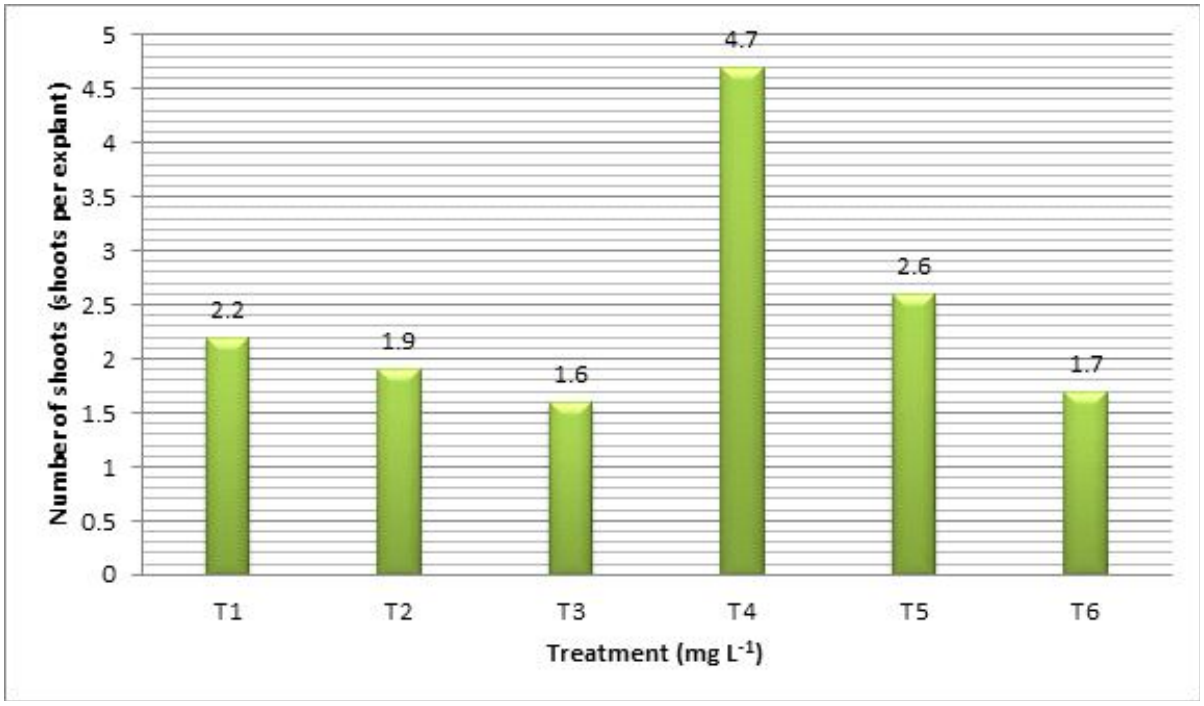

Fig. 2: Effect of different combinations of BA+NAA on the shoot number (R-LSD p ≥ 0.01 = 1.140)

number of shoots reaching 4.7 shoots per explant. While, the treatments (T_9 and T_6) recorded the lowest value in the number of shoots, amounting to 1.7 shoots per explant.

For shoot length, treatment T_4 recorded the highest average length of shoots, which was 1.060 cm, which did not differ significantly from treatment T_5, which recorded an average shoot length of 1.055 cm. While, it was the lowest average of shoot length, which was 0.725 cm in the treatments T_1, T_3 and T_6 (Fig. 3).

Fig. 4 shows that treatments have significant differences in the number of leaves. Treatment T_8 recorded the biggest value of leaves number, amounting to 4.9 leaves per shoot. While the lowest average number of leaves was 2.1 leaves per shoot in treatment T_6.

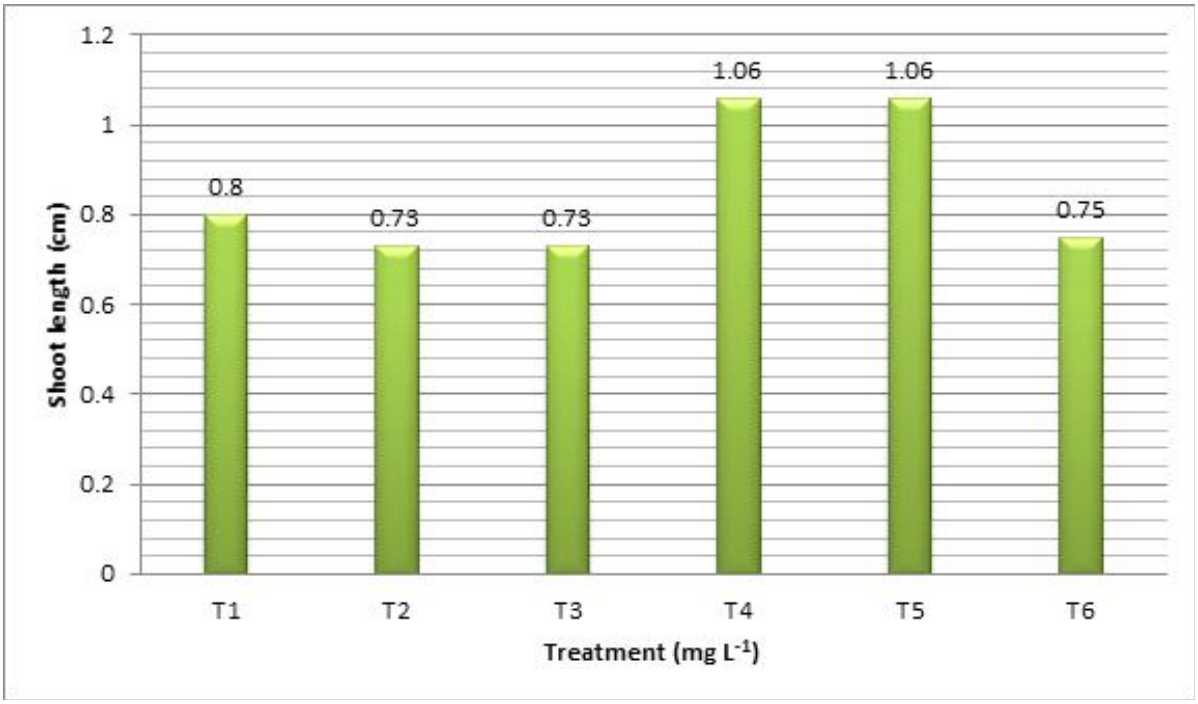

Fig. 3: Effect of different combinations of BA+NAA on the shoot length cm (R-LSD $p \geq 0.01 = 0.240$)

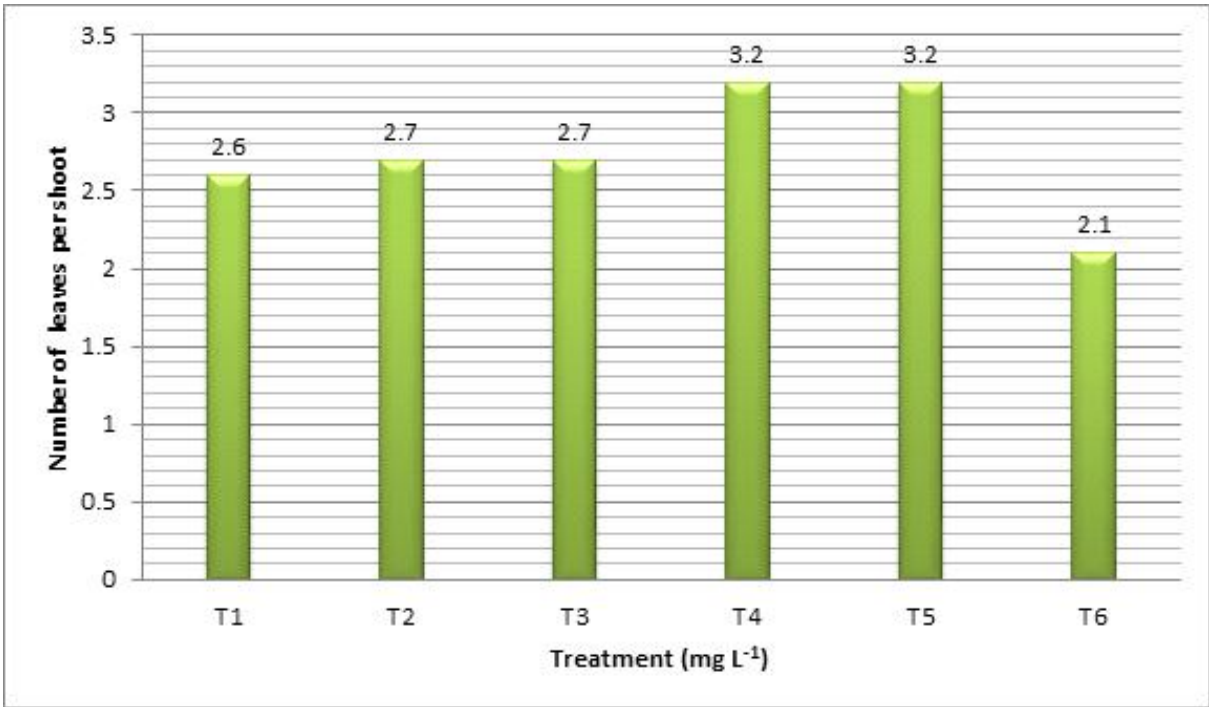

Fig. 4: Effect of different combinations of BA+NAA on the number of leaves per shoot (R-LSD $p \geq 0.01 = 1.08$)

Fig. 5 shows no significant differences in the fresh weight of the shoots between treatments. Fig. 6 indicates that there are significant differences between the treatments in the dry weight of the shoots. All combinations of treatments differ from T_2. The T_7 treatment recorded the highest dry weight of the shoots, amounting to 0.052 g.

These results agreed with Hidaka *et al*. (2008), Ibrahim and Daraj (2015), Setargie *et al*. (2015) and Rohini (2017), who mentioned that an MS medium containing 1.0 mg L^{-1}BA + 0.5 mg L^{-1} NAA (T_4) recorded the highest number of shoot multiplication. The increased concentration of BA and NAA led to a decrease in the number of shoots. This difference in

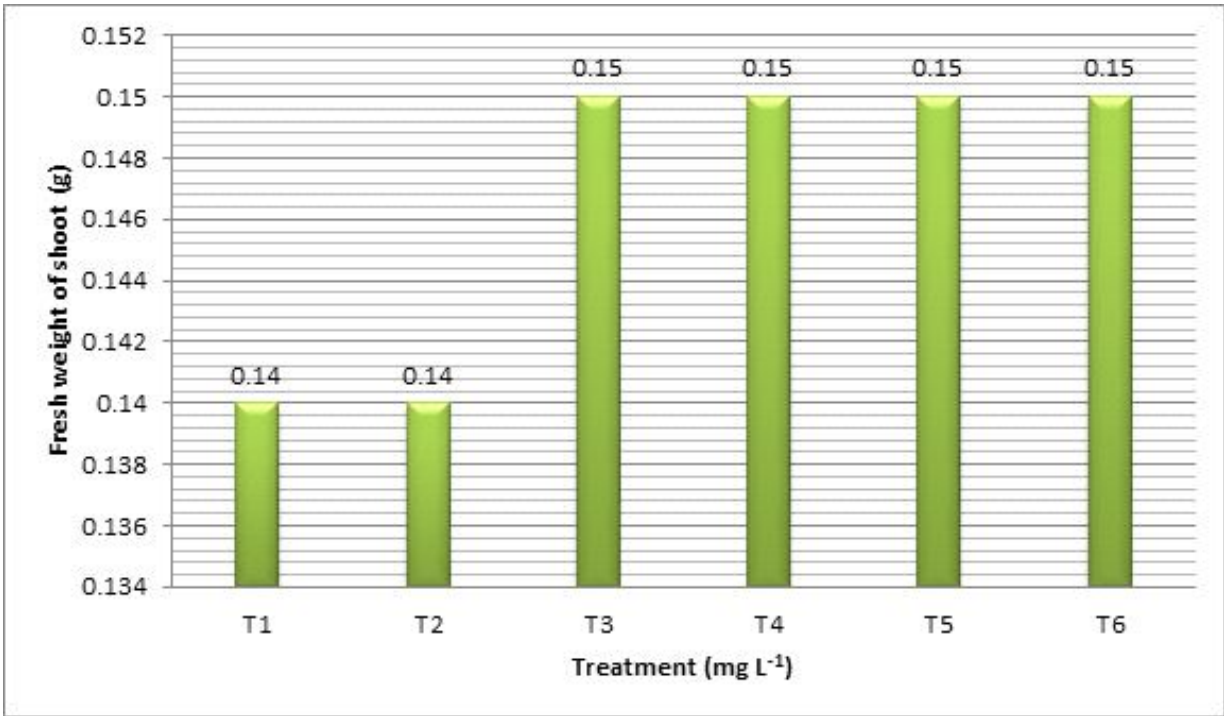

Fig. 5: Effect of different combinations of BA+NAA on the fresh weight of shoot (R-LSD $p \geq 0.01$ = Non significant)

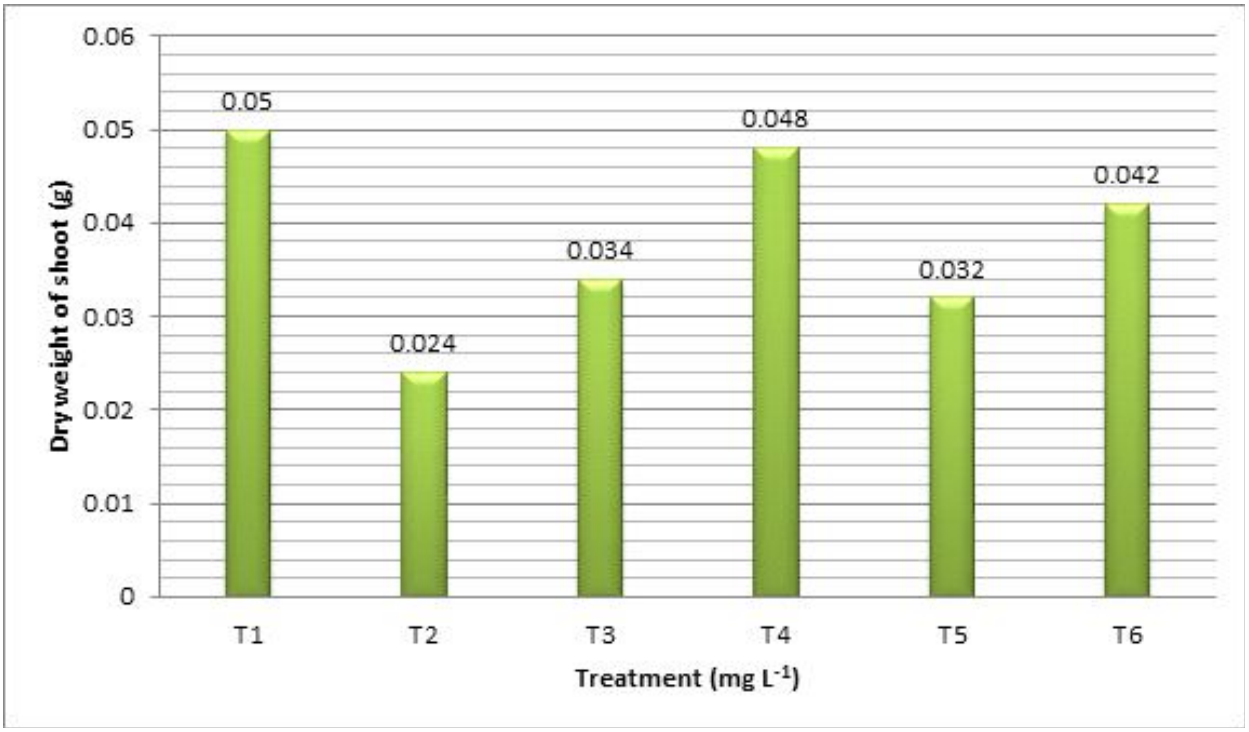

Fig. 6: Effect of different combinations of BA+NAA on the dry weight of shoot (R-LSD $p \geq 0.01 = 0.0269$)

the number of shoots is due to the physiological inhibitory effects of the synthetic cytokinin of BA at higher concentrations [Anandan *et al*. (2011)]. The availability of BA as well as NAA medium of culture leads to the enhancement of the number of shoots produced by the explant, as the cytokinin BA prevent inactivity and the initiation of shoots, while the auxin NAA increases cellular elongation [Panjaitan *et al*. (2007)]. Cytokinins are nitrogenous bases with high molecular weights that cause at low concentrations multiple physiological effects, including promoting cell division, differentiation and bud growth [George *et al*. (2008)]. The hormonal balance between auxins and cytokinins that have been added to the culture medium has an important and

essential role in stimulating the synthesis of proteins, including enzymes and RNA inside cells in plant tissues that stimulate cell division and shoot formation [Taiz and Zeiger (2013)].

4. Conclusion

The best MS has been produced with 1.5 mg L^{-1} BA and 0.5 mg L^{-1} NAA combination treatment, by obtaining the highest percentage of proliferation and number of shoots of the papaya plant by in vitro culture technique.

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